

WHAT IS CLAIMED IS:

1. A method for sequencing a nucleic acid, the method comprising:  
providing one or more nucleic acid anchor primers;  
providing a plurality of single-stranded circular nucleic acid templates;  
annealing an effective amount of the nucleic acid anchor primer to at least one of the single-stranded circular templates to yield a primed anchor primer-circular template complex;  
combining the primed anchor primer-circular template complex with a polymerase to form an extended anchor primer covalently linked to multiple copies of a nucleic acid complementary to the circular nucleic acid template;  
annealing an effective amount of a sequencing primer to one or more copies of said covalently linked complementary nucleic acid;  
extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer, a sequencing reaction byproduct; and  
identifying the sequencing reaction byproduct, thereby determining the sequence of the nucleic acid.
2. The method of claim 1, wherein said anchor primer is linked to a solid support.
3. The method of claim 2, wherein said anchor primer is linked to the solid support prior to formation of said extended anchor primer.
4. The method of claim 2, wherein said anchor primer is linked to the solid support after formation of said extended anchor primer.
5. The method of claim 2, wherein said anchor primer is linked to the solid support during formation of said extended anchor primer.

6. The method of claim 1, wherein the circular nucleic acid template is single-stranded DNA.
7. The method of claim 1, wherein the circular nucleic acid template is an open circle nucleic acid or a closed-circle nucleic acid.
8. The method of claim 1, wherein the circular nucleic acid template is genomic DNA or cDNA.
9. The method of claim 1, wherein the circular nucleic acid is 10-200 nucleotides in length.
10. The method of claim 1, wherein the circular nucleic acid is 20-1000 nucleotides in length.
11. The method of claim 1, wherein the circular nucleic acid is 40-500 nucleotides in length.
12. The method of claim 1, wherein the primed circular template is extended by rolling circle amplification to yield a single-stranded concatamer of the annealed circular nucleic acid template.
13. The method of claim 10, wherein synthesis is carried out with biotin-conjugated nucleotides.
14. The method of claim 10, further comprising:
  - annealing a reverse primer to the single-stranded concatamer to yield a primed concatamer template, and
  - combining the primed concatamer template with a polymerase and nucleotide triphosphates to generate multiple copies of the concatamer template.
15. The method of claim 1, wherein the sequencing byproduct is pyrophosphate.

16. The method of claim 15, wherein the pyrophosphate is detected by contacting the sequencing byproduct with ATP sulfurylase or nicotinamide-mononucleotide adenylyl transferase under conditions sufficient to form ATP.

17. The method of claim 15, wherein the pyrophosphate is detected by contacting the sequencing byproduct with nicotinamide-mononucleotide adenylyl transferase under conditions sufficient to form ATP.

18. The method of claim 16, wherein the sulfurylase is a thermostable sulfurylase.

19. The method of claim 17, wherein the adenylyl transferase is a thermostable adenylylase.

20. The method of claim 16, wherein the ATP is detected by luciferase.

21. The method of claim 20, wherein the luciferase is a thermostable luciferase.

22. The method of claim 15, wherein at least one of the enzymes for detection of pyrophosphate is immobilized on the substrate.

23. The method of claim 15, further comprising apyrase.

24. The method of claim 15, further comprising washing the sequencing product with a wash buffer.

25. The method of claim 24, wherein the wash buffer includes apyrase.

26. The method of claim 1, wherein the anchor primer sequence includes a biotin group.

27. The method of claim 26, wherein the biotin group on the anchor primer is linked to a biotin-binding protein on the solid support.

28. The method of claim 1, wherein one or more of the anchor primers is linked to a polysaccharide.

29. The method of claim 28, wherein one or more of the anchor primers is linked to a plurality of avidin moieties.

30. The method of claim 28, wherein one or more of the anchor primers is linked to a hexahistidine tag and the polysaccharide chain includes a nitrilotriacetic acid (NTA) moiety.

31. The method of claim 28, wherein the polysaccharide is linked to the solid support.

32. The method of claim 15, wherein the ATP sulfurylase or nicotinamide-monomonucleotide adenylyl transferase is linked to a polysaccharide.

33. The method of claim 20, wherein the luciferase is linked to a polysaccharide.

34. The method of claim 32, wherein one or more of the enzymes carry biotin groups and the polysaccharide chain possesses a plurality of avidin moieties.

35. The method of claim 32, wherein one or more of the enzymes carry hexahistidine and the polysaccharide chain possesses a plurality of NTA.

36. The method of claim 32, wherein the polysaccharide chain is linked to the solid support.

37. The method of claim 1, wherein the solid support includes at least one optical fiber.

38. The method of claim 1, wherein the sequencing primer is extended in the presence of a dATP analog.
39. The method of claim 38, wherein the dATP analog is  $\alpha$  thio dATP.
40. The method of claim 1, wherein the solid substrate includes two or more anchor primers separated by approximately 10  $\mu\text{m}$  to approximately 200  $\mu\text{m}$ .
41. The method of claim 1, wherein the solid substrate includes two or more anchor primers separated by approximately 50  $\mu\text{m}$  to approximately 150  $\mu\text{m}$ .
42. The method of claim 40, wherein the solid substrate includes two or more anchor primers separated by approximately 100  $\mu\text{m}$  to approximately 150  $\mu\text{m}$ .
43. The method of claim 1, wherein the solid support matrix comprises a plurality of anchor pads that are linked to the solid support.
44. The method of claim 1, wherein the solid support matrix comprises a plurality of anchor pads that are covalently linked to the solid support.
45. The method of claim 30, wherein the surface area of each anchor pad is approximately 10  $\mu\text{m}^2$ .
46. The method of claim 30, wherein each pad is separated from one another by a distance ranging from approximately 50  $\mu\text{m}$  to approximately 150  $\mu\text{m}$ .
47. The method of claim 1, wherein said method comprises sequencing at least 100 nucleic acids.

48. The method of claim 1, wherein said method comprises sequencing at least 1000 nucleic acids.

49. The method of claim 1, wherein said method comprises sequencing at least 10,000 nucleic acids.

50. A method for analyzing a mixture of polymers, the method comprising:  
providing a plurality of at least 1,000 independent reaction sites attached to a solid substrate, wherein said independent reaction sites comprise a reaction center and said independent reactions sites are separated by distances that eliminate diffusion of reactants and products between sites;

contacting said plurality of reaction sites with a fluid comprising said mixture of polymers; and

separating said polymers from the fluid and from each other by attachment to the reaction sites, thereby analyzing said mixture of biopolymers.

51. The method of claim 50, wherein said polymer is a biopolymer.

52. The method of claim 50, wherein said polymer is DNA, RNA, or a polypeptide.

53. The method of claim 50, wherein said medium is a low diffusivity medium.

54. The method of claim 50, wherein said biopolymers are separated by electrophoresis.

55. The method of claim 50, wherein said reaction sites are disposed on a planar surface.

56. A substrate for analyzing a nucleic acid, the substrate comprising:  
a cavitated fiber optic surface; and  
a nucleic acid sequence linked to the fiber optic surface.

57. The substrate of claim 56, wherein the nucleic acid sequence is an anchor primer.
58. The substrate of claim 56, wherein the substrate comprises a plurality of fiber optic surfaces.
59. The substrate of claim 58, wherein the fiber optic surface includes two or more anchoring primers separated by approximately 10  $\mu\text{m}$  to approximately 200  $\mu\text{m}$ .
60. The substrate of claim 58, wherein the fiber optic surface includes two or more anchoring primers separated by approximately 100  $\mu\text{m}$  to approximately 150  $\mu\text{m}$ .
61. The substrate of claim 58, wherein the fiber optic surface includes two or more anchoring primers separated by approximately 150  $\mu\text{m}$ .
62. The substrate of claim 58, wherein the fiber optic surface includes two or more anchor pads separated by approximately 100  $\mu\text{m}$  to approximately 150  $\mu\text{m}$ .
63. The substrate of claim 62, wherein the surface area of each pad is approximately 10  $\mu\text{m}^2$ .
64. ~~A substrate with a cavitated surface comprising  $10^3$  or more groups of oligonucleotides attached to the surface in discrete known regions, the  $10^3$  or more groups of oligonucleotides occupying a total area of less than 1  $\text{cm}^2$  on said substrate, said groups of oligonucleotides having different nucleotide sequences.~~
65. The substrate of claim 64, wherein said substrate comprises  $10^4$  or more different groups of sequences in discrete known regions.
66. The substrate of claim 64, wherein said substrate comprises  $10^5$  or more different groups of oligonucleotides with known sequences in discrete known regions.

67. The substrate of claim 64, wherein the groups of oligonucleotides are attached to the surface by a linker.

68. The substrate of claim 64, wherein the groups of oligonucleotides are covalently attached to the surface.

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69. An array of more than 1,000 different groups of oligonucleotide molecules with known sequences covalently coupled to a surface of a cavitated substrate, said groups of oligonucleotide molecules each in discrete known regions and differing from other groups of oligonucleotide molecules in monomer sequence, each of said discrete known regions being an area of less than about  $0.01 \text{ cm}^2$  and each discrete known region comprising oligonucleotides of known sequence, said different groups occupying a total area of less than  $1 \text{ cm}^2$ .

70. The array of claim 69, wherein said area is less than  $10,000 \text{ microns}^2$ .

71. The array of claim 69, wherein said array is made by the process of:

exposing a first region of said substrate to light to remove a photoremovable group from photoprotected first region, and not exposing a second region of said surface to light; chemical activation of deprotected areas, covalently coupling a first nucleotide to said activated areas, applying a new mask to deprotect areas not deprotected in the first step, repeating the nucleotide coupling step thus covalently coupling a second nucleotide to said region exposed to light; and repeating said steps of exposing said substrate to light and covalently coupling nucleotides until more than 100 different groups of nucleotides are formed on said surface.

72. The array of claim 69, wherein said array comprises more than 10,000 groups of oligonucleotides of known sequences.

73. An apparatus for analyzing a nucleic acid sequence, the apparatus comprising:



a reagent delivery chamber, wherein the chamber includes a substrate with immobilized nucleic acids;

a conduit in communication with the reagent delivery chamber;

an imaging system in communication with the reagent delivery chamber; and

a data collection system in communication with the imaging system.

74. The apparatus of claim 73, wherein the substrate is a planar substrate.
75. The apparatus of claim 73, wherein the substrate is a cavitated planar substrate.
76. The apparatus of claim 73, wherein the imaging system is a fiber optic system.
77. The apparatus of claim 73, wherein the substrate comprises  
a cavitated fiber optic surface in communication with said imaging system; and  
a nucleic acid sequence linked to the fiber optic surface.
78. The apparatus of claim 77, wherein the substrate comprises a plurality of fiber optic surfaces, said fiber optic surfaces being in communication with said imaging system.
79. The apparatus of claim 78, wherein the fiber optic surfaces include two or more anchoring primers separated by approximately 100  $\mu\text{m}$  to approximately 150  $\mu\text{m}$ .
80. The apparatus of claim 78, wherein the fiber optic surfaces include two or more anchoring primers separated by approximately 150  $\mu\text{m}$ .
81. The apparatus of claim 78, wherein the fiber optic surfaces include two or more anchor pads separated by approximately 100  $\mu\text{m}$  to approximately 150  $\mu\text{m}$ .
82. The apparatus of claim 78, wherein the surface area of each pad is approximately 5  $\mu\text{m}^2$  to approximately 10,000  $\mu\text{m}^2$ .

83. The apparatus of claim 78, wherein the surface area of each pad is approximately  $10 \mu\text{m}^2$ .

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84. An apparatus for processing a plurality of analytes, the apparatus comprising:  
a flow chamber having disposed therein a substrate comprising a plurality of cavitated surfaces, said cavitated surfaces having disposed thereon nucleic acid molecules;  
fluid means for delivering processing reagents from one or more reservoirs to the flow chamber so that the analytes anchored to the plurality of microparticles are exposed to the reagents; and  
detection means for detecting a sequence of optical signals from each microparticle of the plurality, each optical signal of the sequence being indicative of an interaction between a processing reagent and the analyte anchored thereto, wherein said detection means is in communication with the cavitated surfaces.

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85. The apparatus of claim 85, wherein said detection means further comprises signal tracking means for correlating said optical signals from each of said microparticles in each of said digital images to form for each said microparticle of said plurality a sequence of said optical signals.

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86. The apparatus of claim 87, wherein said signal tracking means is a CCD camera.

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87. The apparatus of claim 86, wherein said analyte is DNA.